Ubiquitin-dependent control of hESC self-renewal and expansion

Grant Award Details

Ubiquitin-dependent control of hESC self-renewal and expansion

Grant Type: Basic Biology III

Grant Number: RB3-02222

Project Objective: The overall objective is to explore ubiquitin-dependent mechanisms of hESC division, the insights from which could enable more faithful expansion of hESC and elimination of undifferentiated cells.

Investigator:

Name:	Michael Rape
Institution:	University of California, Berkeley
Type:	PI

Human Stem Cell Use: Embryonic Stem Cell

Award Value: $1,224,805

Status: Closed

Progress Reports

Reporting Period: Year 1

View Report

Reporting Period: Year 2

View Report

Reporting Period: Year 3

View Report

Grant Application Details

Application Title: Ubiquitin-dependent control of hESC self-renewal and expansion

Public Abstract:

Human embryonic stem cells (hESCs) or induced pluripotent stem cells (iPSCs) provide an invaluable resource for regenerative medicine and disease modeling. To be able to use these cells in the clinic, hESCs and iPSCs need to be expanded without introducing genetic instability. However, current protocols of hESC and iPSC propagation frequently result in aneuploidy, a potentially tumorigenic cell state. Because of their tumorigenic potential, undifferentiated hESCs have to be removed from cell populations prior to transplantation, yet efficient ways to securely achieve this have not been developed. Together, these limitations greatly limit the use of hESCs or iPSCs in regenerative medicine.

Here, we propose to dissect and manipulate mechanisms of hESC division and survival. Based on our preliminary data and previous observations in embryonic cells, we will initially dissect the role and regulation of the anaphase-promoting complex (APC/C), an essential component of the core cell cycle machinery, and Cul3, an enzyme required for the integration of extracellular signaling into the hESC division program. These experiments will make use of our experience in developing biochemical systems to dissect complex pathways in vitro, combined with an in-depth analysis of cell cycle control in hESCs in vivo. Understanding hESC division control by APC/C and Cul3 will identify the mechanisms generating aneuploidy during hESC culture. Subsequently, we will isolate novel hESC-specific ubiquitination enzymes required for division and survival by using siRNA screens in hESCs. We will identify the substrates of critical enzymes to determine their role in division and survival control. Manipulating the activity of hESC-specific enzymes or substrates will allow the removal of undifferentiated cells from cell populations, an essential step prior to transplantation.

The results from these studies will provide critical insight into the mechanisms controlling hESC division and survival. Based on findings on division control, we will be able to develop protocols for faithful hESC or iPSC expansion in culture, while understanding the mechanisms of hESC survival will point to strategies for the selective elimination of undifferentiated cells from cell populations. Both outcomes of this study will greatly expand the use of stem cells for regenerative medicine.

Statement of Benefit to California:

Human embryonic stem cells (hESCs) or induced pluripotent stem cells (iPSCs) provide an invaluable resource for regenerative medicine and disease modeling. To be able to use these cells in the clinic, hESCs and iPSCs need to be expanded without introducing genetic instability. However, current protocols of hESC and iPSC propagation frequently result in aneuploidy, a potentially tumorigenic cell state. Because of their tumorigenic potential, undifferentiated hESCs have to be removed from cell populations prior to transplantation, yet efficient ways to securely achieve this have not been developed. Together, these limitations greatly limit the use of hESCs or iPSCs in regenerative medicine.

Here, we propose to dissect and manipulate mechanisms of hESC division and survival. We will identify and dissect the mechanisms that control the core hESC division machinery and those that achieve the integration of extracellular signals into the hESC division program. Findings from these studies will allow us to develop protocols for the faithful expansion of hESCs or iPSCs in culture, an essential step for using these cell types for differentiation or as disease models. In the last part of this work, we will use siRNA screens to isolate novel ubiquitination enzymes and their substrates that are required for hESC division and survival. Manipulating the activity of these proteins will provide strategies for eliminating undifferentiated cells from cell populations. As current differentiation protocols are inefficient, the selective removal of undifferentiated cells is required before transplantation of differentiated cells can occur. The results from this part of our study can also be implemented to selectively kill de-differentiated tumor cells during chemotherapy.

Together, our study will provide critical insight into the mechanisms controlling hESC division and survival to develop protocols for faithful hESC or iPSC expansion in culture and for the selective elimination of undifferentiated cells from cell populations. Both outcomes of this study can be directly translated for applications in the clinic, greatly expanding the use of stem cells for regenerative medicine.
